Reply to E. Lax and D.M. Sapozhnikov

Emily Jobe[1,2] and Yu Gao[2,3]

(Edited by Xinyu Zhao[1,2,3])

[1]Cellular and Molecular Biology Graduate Program, [2]Waisman Center, and [3]Department of Neuroscience, University of Wisconsin-Madison, Madison, Wisconsin 53705,

We appreciate the interest for our study on Mbd1 regulation of adult neurogenesis. Lax and Sapozhnikov raise good questions, which we have considered or are currently working on. We would like to offer some clarifications and responses to the issues they have raised.

First as regards the statement “MBD1 was expressed in NSCs and adult neurons but not in immature neurons or astrocytes”, we would like to clarify that we did find MBD1 expressed in some DCX positive immature neurons. Overall, MBD1 cannot be detected by staining at an early DCX stage, but is expressed in other DCX positive cells, which are generally considered immature neurons.

Second, regarding the potential changes in epigenetic marks of the beta-gal reporter, we do understand these concerns; however, it is unlikely that the epigenetic modification of the Mbd1 promoter would change given the following. (1) Mbd1 is not an imprinted gene, which is subject to epigenetic inheritance. Thus, even if the Mbd1 promoter on the targeting vector does not contain any epigenetic marks, after targeting and successive cell divisions and generations of the line, the epigenetic marks would be reprogrammed to normal state. (2) We found a reduction of MBD1 expression in the early stage of DCX+ cells, which might suggest that MBD1 expression is regulated dynamically by epigenetic mechanisms. (3) According to our RNA-seq data, we did not find any genes near to Mbd1 that were differentially expressed between Mbd1 KO and WT, suggesting that modification of the Mbd1 CDS sequence does not induce epigenetic change on adjacent genes.

Third, we appreciate that the authors discussed potential hypothesis to address the fact that some genes were down-regulated in Mbd1 KO cells even though Mbd1 has been characterized as a gene repressor. We think this question is interesting too! In fact, we have just completed another study which investigates some of these issues. We agree that to confirm the precise regulatory mechanism of MBD1, a combination of ChIP-seq and RNA-seq is required. In our upcoming study, we combined ChIP-seq and RNA-seq from dentate gyrus tissue and we have some interesting findings, so please keep an eye out for our next publication!

As a final note, in the first sentence of this journal club manuscript, the authors wrote “The generation of new neurons in the sub-ventricular zone of the dentate gyrus during adulthood is indispensable to hippocampal- dependent learning and memory formation (Gonc alves et al., 2016).” Subventricular zone should be changed to subgranular zone.